(91C97)
F121

San Joaquin River Drainage Fall-Run Chinook Salmon Genetic Baseline and Discrimination Evaluation

II. Title Page

Applicant: California Department Fish and Game
Region 4
1234 E. Shaw Ave.
Fresno, CA 93710
Phone: (209) 243-4005
FAX: (209) 243-4022

Type Organization: Public Agency

Contact Person: Mr. W. George Neillands
P.O. Box 10
La Grange, CA 95329
Phone: (209) 853-2534
FAX: (209) ~~853-2536~~ (209) 853-9017

Collaborators: University of California

RFP Project Type: Other Service (Monitoring)

F1-121

San Joaquin River Drainage Fall-Run Chinook Salmon Genetic Baseline and Discrimination Evaluation

I. Executive Summary

Applicant Name: California Department of Fish and Game
Region 4
1234 E. Shaw Ave.
Fresno, CA 93710

Project Description and Primary Biological/Ecological Objectives

A detailed understanding of the genetic integrity and relatedness of stocks in decline is of crucial importance for the planning of effective ecosystem restoration measures. We are proposing to create a comprehensive genetic archive, and thoroughly describe the genetic variability of chinook salmon between and within streams in the San Joaquin River basin. We propose a three year evaluation to characterize and discriminate to the extent feasible the genetic makeup of fall-run chinook salmon in the tributaries of the San Joaquin River relative to those in the Sacramento River basin. This archive will then be used as a "genetic baseline" by which all subsequent ecosystem restoration and enhancement efforts in the Central Valley can be compared. The results of this proposed project would be useful in stock management decisions ranging from population, habitat or harvest management to hatchery operations.

Approach/Tasks/Schedule

We propose a three year evaluation to characterize and discriminate to the extent feasible the genetic makeup of fall-run chinook salmon in the tributaries of the San Joaquin River relative to those in the Sacramento River basin. Similar to recent genetic work performed on Sacramento winter-run and spring-run stocks, this evaluation would develop information from 50+ known microsatellites and use a screening process to discriminate this fall-run stock from other. The first year would begin development of an extensive genetic tissue archive and develop preliminary information on the microsatellites and buffers of greatest utility. This would be followed by two years of specific analyses, genetic characterization and discrimination techniques.

Justification for Project and Funding by CALFED

Genetic factors have been identified by the CALFED Bay-Delta Program as an important population management stressor related to artificial propagation of fish. Artificial propagation has significant genetic and management implications. In addition, other population management stressors, such as water management activities, may also result in long-term genetic and ecological changes. We are proposing to augment current genetic investigations related to winter-run and spring-run chinook salmon stocks in the Central Valley to improve monitoring, sampling, and our ability to resolve mixtures of gene pools within the San Joaquin River basin.

Banks et al. (1996) revealed six highly polymorphic microsatellite loci which indicated a strong potential for stock discrimination. Significant allele frequency differences between the various chinook salmon runs in the upper Sacramento River demonstrated that although the same alleles are common in more than one stock, allele and genotypic frequency profiles show strong stock dependency. They recommend the isolation and characterization of more microsatellite loci for chinook salmon to increase the power for stock discrimination. These studies to date have generally included small samples from within the San Joaquin River basin. with the majority of these samples from the Merced River Hatchery.

Budget Costs and Third Party Impacts

The development of the Mixed Stock Analysis of Central Valley chinook salmon has been partially funded by DWR, and partially CALFED Category III funding (spring-run project). We plan on coordinating with these agencies on the Mixed Stock Analysis of Central Valley chinook salmon and plan on negotiating an interagency agreement with the University of California to complete the scope of work. The estimated cost to implement this project is approximately $387,003 over three years.

Applicant Qualifications

The DFG Region 4's anadromous fisheries staff have worked closely with various other state, federal and private personnel, to construct and repair chinook salmon spawning, and rearing habitat in the San Joaquin River basin. The DFG has the clerical, fiscal and contractual personnel necessary to support the biological and technical experts administering this project. The DFG would also utilize information and recommendations obtained from the Genetic Review Committee associated with the proposed Tuolumne River Hatchery planning process.

Monitoring and Data Evaluation

Given the potential for genetic variance among different year classes, particularly for fall-run populations of small effective size, it is important to sample populations over a three year period. Year one (1997-98) will involve further development of microsatellites found to be informative for discrimination between relevant fall-run populations in comparison to each other as well as in comparison to other runs. Year two (1998-99) will continue development of microsatellite resources for run discrimination but will also focus on alternate techniques for increased efficiency in population characterization, automation of procedures and the refinement of statistical and computing resources. Year three (1999-2000) will concentrate on the synthesis of a complete data set for fall-run populations taken all three years but concentrating on those microsatellites and techniques demonstrated to have the greatest power for identification of genetic integrity and population discrimination.

Local Support/Coordination with other Programs/Compatibility with CALFED Objectives

This project is supported by the Genetic Review Committee associated with the proposed Tuolumne River Hatchery planning process, the Tuolumne River Technical Advisory Committee. and San Joaquin River basin stakeholders. DWR and IFD have already implemented similar projects in the upper Sacramento River basin and this proposed project would complement their efforts.

III. Project Description

Project Description and Approach

A detailed understanding of the genetic integrity and relatedness of stocks in decline is of crucial importance for the planning of effective ecosystem restoration measures. In addition, genetic identification of chinook salmon to run at particular localities in the river, or possibly even in the ocean, will enable modification of water diversion and/or fishing strategies in order to protect particular runs. There are four primary genetic risks associated with any ecosystem restoration or enhancement project; they are 1) extinction, 2) loss of genetic variability, 3) inbreeding, and 4) 'outbreeding depression" (or loss of genetic fitness). The risk of extinction is currently being monitored through the Department's San Joaquin River basin fall-run chinook salmon annual escapement surveys. We are proposing to create a comprehensive genetic archive, and thoroughly describe the genetic variability of chinook salmon between and within streams in the San Joaquin River basin. This archive will then be used as a "genetic baseline" by which all subsequent ecosystem restoration and enhancement efforts in the Central Valley can be compared. The results of this proposed project would be useful in stock management decisions ranging from population, habitat or harvest management to hatchery operations. The tissue archive will be made available for future analysis as the technology to detect genetic change becomes more advanced. We are currently reviewing literature and will be developing pilot programs to measure the risk due to inbreeding and the risk due to loss of genetic fitness associated with current artificial propagation efforts.

We propose a three year evaluation to characterize and discriminate to the extent feasible the genetic makeup of fall-run chinook salmon in the tributaries of the San Joaquin River relative to those in the Sacramento River basin. Similar to recent genetic work performed on Sacramento winter-run and spring-run stocks, this evaluation will develop information from 50+ known microsatellites and use a screening process to discriminate this Fall-run stock from other. The first year will begin development of an extensive genetic tissue archive and develop preliminary information on the microsatellites and buffers of greatest utility. This will be followed by two years of specific analyses, genetic characterization and discrimination techniques.

Our goals for this proposal are fourfold. First, we will create a comprehensive collection of tissue samples for genetic evaluations and thoroughly characterize the existing diversity of fall-run chinook salmon in the San Joaquin River basin, relative to winter-run, spring-run and other fall-runs in the Central Valley. We will include genetic tissue samples from chinook salmon in the San Joaquin River tributaries to evaluate the genetic variation associated with their spatial, temporal, and age distributions in the annual escapements. Secondly, we will address automation of molecular genetic techniques for DNA extraction, amplification and characterization such that we will be able to accumulate microsatellite·

frequency data for San Joaquin River basin chinook salmon. Thirdly, we will develop statistical and computing resources for efficient synthesis of data and drawing inference for characterization of run integrity and discrimination. Fourth, we will investigate alternate techniques in molecular genetics that show potential for even greater efficiency for matching the rapid turnaround required by management needs.

Location and/or Geographic Boundaries of Project

The primary focus of this project is fall-run chinook salmon in the San Joaquin River and its tributaries. The major eastside tributaries south of the Delta supporting fall-run chinook salmon spawning and rearing are the Stanislaus, Tuolumne, and Merced rivers. The majority of the proposed field work would be conducted within Stanislaus and Merced counties in the San Joaquin River basin. The fall-run chinook salmon race in the San Joaquin River is designated as a species of concern by USFWS. The secondary focus for this project will be East Side Delta tributaries, and Sacramento River basin tributaries.

Expected Benefit(s)

This information would help in further characterizing the chinook salmon stocks in the San Joaquin Valley and further define the stocks throughout the Central Valley. Discrimination of San Joaquin fall-run stock from other fall-runs in the Central Valley will be difficult based on our knowledge to date. However, even if reliable discrimination is not accomplished, we will have characterized the genetic nature of the existing stock as thoroughly as possible and created an archive of San Joaquin fall-run chinook salmon tissue for future use. This information would form the basis (baseline) from which subsequent management practices are measured. Such as the Merced River Hatchery, the proposed Tuolumne River Hatchery, angling regulations, protective water quality standards, water project operations, long term CALFED projects, habitat restoration projects, etc.. Ecosystem-level changes may influence the selective pressures on San Joaquin fall-run stock, resulting in long-term changes in genetic character. A sound baseline at the beginning of the program would provide a necessary "yardstick" for the evaluation of future changes in genetic variation.

Assessing the genetic integrity of natural spawners in the San Joaquin River basin would help identify and prevent any further unintentional hybridization between alternate runs. It would also help identify and avoid the potential loss of genetic fitness associated with supplementation efforts in the basin. Genetic discrimination between alternate natural runs and those derived from hatcheries is of increasing importance. Accurate estimates of natural run effective population size in comparison to that of supplementation or hatchery stocks would avoid reduction of genetic diversity of natural spawners. Escapement surveys and estimates of effective population size based on juveniles captured during outmigration may provide the best means for measuring long-term ecosystem restoration efforts upstream.

Background and Biological/Technical Justification

Genetic factors have been identified by the CALFED Bay-Delta Program as an important population management stressor related to artificial propagation of fish. Artificial propagation has significant genetic and management implications, in addition to other population management stressors, such as water management activities that may result in migratory pathway changes. We are proposing to augment current genetic investigations related to winter-run and spring-run chinook salmon stocks in the Central Valley to improve monitoring, sampling, and our ability to resolve mixtures of gene pools within the San Joaquin River basin.

Current studies have taken advantage of the ability to directly examine DNA samples, first from mitochondrial DNA (mtDNA) and then, as molecular techniques developed, nuclear DNA (nDNA). Neilsen (1994) evaluated mitochondrial DNA and nuclear DNA microsatellite polymorphisms in chinook salmon samples from the Sacramento-San Joaquin River basin. Small but significant haplotypic frequency differences were detected between two successive years for returning adults at one of two hatcheries, and some fall-run natural samples were significantly different from frequencies in samples from fall-run hatchery samples. Hedgecock et al (1995) identified polymorphisms at microsatellite loci which distinguished between the various runs in the upper Sacramento River. Banks et al. (1996) revealed six highly polymorphic microsatellite loci which indicated a strong potential for stock discrimination. Significant allele frequency differences between the various chinook salmon runs in the upper Sacramento River were identified. They have demonstrated that although the same alleles are common in more than one stock, allele and genotypic frequency profiles show strong stock dependency. They recommend the isolation and characterization of more microsatellite loci for chinook salmon to increase the power for stock discrimination. These studies have generally included small samples from within the San Joaquin River basin, mostly samples from the Merced River Hatchery.

A Genetic Review Committee (associated with the Tuolumne River Hatchery planning effort) recommended a three year evaluation to establish a genetic "baseline" and attempt discrimination with the best methods currently available. The results of this proposed project would be useful in long-term stock management decisions ranging from population, habitat or harvest management to hatchery operations.

At least five different molecular methods might be proposed to tackle the problem of Central Valley chinook stock identification at the nDNA level: (1) multi- and single-locus minisatellites ; (2) RAPDs (randomly amplified polymorphic DNA;), (3) restriction fragment length polymorphisms (RFLP) of annonymous single-copy nDNA, (4) RFLP of gene introns; and (5) microsatellites or STRPs. Banks et al. (1996) chose microsatellites over alternative methods because they were successfull at isolating microsatellites which characterized and distinguished runs in the upper Sacramento River basin.

Microsatellites are chosen over alternative methods because: (1) they can be amplified by the polymerase chain reaction (PCR), allowing non-destructive sampling of fin clips for genetic analysis; (2) provide abundant information for what are very likely selectively neutral polymorphisms; (3) variation at microsatellite loci is codominantly inherited, so that alleles and genotypes at defined Mendelian loci are readily and reliably scored; (4) once they are isolated, cloned, sequenced, and made amenable to PCR amplification from crude tissue extracts, they are easy to score in large population surveys.

Proposed Scope of Work

The objectives of the proposed research are:

(1) to develop analytical methods for revealing short tandem repeat nDNA polymorphisms (STRPs) in San Joaquin chinook salmon;

(2) to acquire baseline data on the frequencies of alleles at these polymorphic marker loci in as many of the San Joaquin chinook salmon populations as possible;

(3) to look for fixed, diagnostic STRP differences among spawning stocks, but failing that;

(4) to develop statistical methods for estimating, from non-fixed genetic differences, the relative contributions of these stocks to catches of juveniles in the Delta;

(5) to validate the Mixed Stock Analysis (MSA) by sampling coded-wire tagged hatchery releases of fall-run chinook salmon.

Given the potential for genetic variance among different year classes, particularly for fall-run populations of small effective size, it would be important to sample populations over a three-year period. Year one (1997-98) would involve further development of microsatellites found to be informative for discrimination between relevant fall-run populations in comparison to each other as well as in comparison to other runs. We plan to conclude with a preliminary report for loci and techniques developed by this time. Year two (1998-99) would continue development of microsatellite resources for run discrimination but will also focus on alternate techniques for increased efficiency in population characterization, automation of procedures and the refinement of statistical and computing resources. A preliminary report for loci, alternate techniques, automation procedures, statistical procedures and interim costs to date will be developed. Year three (1999-2000) would concentrate on the synthesis of a complete data set for fall-run populations taken all three years but concentrating on those microsatellites and techniques demonstrated to have the greatest power for identification of genetic integrity and population discrimination. The project would be concluded with a final report stating conclusions, recommendations and costs.

Monitoring and Data Evaluation

The mixed stock analysis (MSA) of juvenile chinook salmon in the Sacramento-San Joaquin Delta requires: (1) reasonable understanding of all spawning populations that contribute to the Delta's mixed-stock juvenile populations; (2) identification, if possible, of fixed nDNA differences among the contributing spawning populations; (3) in the absence of fixed differences, statistically reliable baseline data on frequencies of alleles and genotypes for multiple, non-fixed nDNA markers in these spawning stocks; (4) similar, reliable genetic information for samples of juveniles in the Delta; and (5) statistical methods for estimating the contributions of various stocks to juvenile chinook in the Delta, should simple diagnostic differences among San Joaquin River basin spawning stocks not be found.

We are proposing development of San Joaquin River basin chinook salmon microsatellite data set through an interagency agreement with the University of California system, similar to the winter-run and spring-run projects. We are proposing DFG continue to collect and augment tissue samples from natural and hatchery marked fish through current San Joaquin River basin escapement and juvenile surveys, and include samples from their temporal, spatial, and age distributions. The tissue sampling, collection, and storage would be coordinated with Inland Fisheries Division (IFD) staff and DWR personnel. The fall-run San Joaquin River chinook salmon tissue archive would be available for future genetic studies. The contractor would provide written progress reports at approximately six month intervals and oral briefings to an interagency review panel at least once per year and (including possibly the Genetic Review Committee assembled for the proposed Tuolumne River Hatchery planning process.

Implementability

The California Department of Fish and Game (DFG) and the Department of Water Resources (DWR) are currently coordinating the tissue collection from chinook salmon stocks throughout the Central Valley. We would continue to coordinate field collection and the genetic tissue sample archive with IFD staff. We are proposing only to augment the field collection of chinook salmon tissues throughout the San Joaquin River basin and provide the funding for developing the genetic evaluation. DWR is currently developing the microsatellite data set through an interagency agreement with the University of California system. We would propose to use the same approach to facilitate the coordination and development of all San Joaquin River chinook salmon genetic samples.

San Joaquin River Drainage Fall-Run Chinook Salmon Genetic Baseline and Discrimination Evaluation

IV. Costs and Schedule to Implement Proposed Project

Budget Costs

Funding for the Winter-run project involves a number of different Local, State and Federal funding agencies. The development of the Mixed Stock Analysis of Central Valley chinook salmon has been partially funded by DWR, and partially CALFED Category III funding (Spring-run project). We plan on coordinating with these agencies on the Mixed Stock Analysis of Central Valley chinook salmon and plan on negotiating an interagency agreement with the University of California to complete the scope of work.

Project Costs and Funding Partners

		Category III		
YEAR	DWR	Spring-run	SJR Fall-run	TOTAL
1997-98	$200,000	$250,000	$129,000	$579,000
1998-99	$200,000	$100,000	$129,000	$429,000
1999-2000	$200,000	$100,000	$129,000	$429,000
Totals	$600,000	$450,000	$387,003	$1,437,000

	Category III Funds Requested		
	'97-98	'98-99	'99-00
INTERAGENCY AGREEMENT COSTS			
Senior Personnel	$0	$0	$0
Other Personnel	$37,888	$37,888	$37,888
Staff Benefits	$5,959	$5,959	$5,959
SALARIES + BENEFITS	$43,847	$43,847	$43,847
OPERATING EXPENSES	$50,000	$50,000	$50,000
TOTAL DIRECT COSTS	$93,847	$93,847	$93,847
INDIRECT COSTS (@ .10)	$9,354	$9,354	$9,354
PERMENANT EQUIPMENT	$0	$0	$0
DIRECT & INDIRECT COSTS	**$103,201**	**$103,201**	**$103,201**
DFG INDIRECT COSTS (@ .25)	$25,800	$25,800	$25,800
TOTAL DIRECT & INDIRECT COSTS	**$129,001**	**$129,001**	**$129,001**

Schedule Milestones

The following is an outline of the necessary tasks:

TASKS	YEAR		
	1 1997-1998 ONDJFMAMJJAS	2 1998-1999 ONDJFMAMJJAS	3 1999-2000 ONDJFMAMJJAS

1. Clone, sequence, and determine polymorphism of microsatellite loci

a) screen genomic library O----M

b) sequence and develop primers N-------M

c) PCR & survey polymorphism J----------S

2 Collect baseline frequency data for San Joaquin spawning stocks

a) identify & sample stocks O---J O---J O---J

b) PCR & genotype stocks J----------S J----------S J----------S

3 Collect frequency data for outmigrating juveniles in the Delta

a) sample juveniles J-------J J-------J J-------J

b) PCR & genotype juveniles J----------S

4 Conduct a preliminary mixed stock analysis

a) develop estimators J----------M

b) carry out first analysis J----------S

5 Conduct refined mixed stock analysis and verification

a) refine estimators J----------S

b) verify method for tagged hatchery stocks M----------S

Contractor would provide written progress reports at approximately six month intervals and oral briefings to an interagency review panel at least once per year as well as the Genetic Review Committee assembled for the proposed Tuolumne River Hactery planning process..

Third Party Impacts

The only third party impacts foreseen at this point will be negotiating an Interagency Agreement with the University of California. Other parties, Local, State, and Federal that are involved with the MSA will benefit from the additional data.

V. Applicant Qualifications

DFG's Region 4 anadromous fishery staff administered $1.5 million dollars in the 1995-96 fiscal year. In 1995-96 they helped develop 21 habitat restoration projects and completed the environmental documentation for 5 of these projects. They have been named contract managers for several restoration, revegetation, fish screening and fish research projects. Region 4 staff has work closely with the various other state, federal and private personnel, to construct chinook salmon spawning, rearing, predator pond isolation project in the San Joaquin River basin.

The DFG Region 4 staff assigned to implement the San Joaquin River Drainage Fall-Run Chinook Salmon Genetic Baseline and Discrimination Evaluation are:

Mr. Bill Loudermilk, Senior Fisheries Biologist (M/F). Mr. Loudermilk will be responsible for the overall project including supervision, budgeting and contracting.

Mr. W. George Neillands, Associate Biologist (M/F). Mr. Neillands will assist in these responsibilities. He will develop and obtain the necessary Interagency Agreement to implement this project. He will be responsible for the coordination and delivery to IFD of the comprehensive field collection of genetic tissue samples.

This core staff will obtain administrative support from both Region 4's and Inland Fisheries Division's (IFD) clerical, fiscal and contractual personnel. The Genetic Review Committee associated with the proposed Tuolumne River Hatchery will provide technical and scientific review when necessary.

VI. Compliance with Standard Terms and Conditions

DFG is a public agency and will comply with appropriate terms and conditions pursuant to policy, regulation, or law.

Bibliography

Banks, Michael A., B.A. Baldwin, and D. Hedgecock. 1996. Research on Chinook Salmon (*Oncorhynchus tshawytsctha*) Stock Structure Using Microsatellite DNA. Bull. Nat'l. Res. Inst. Aquacult., Suppl. 2:5-9.

Hedgecock, Dennis, M.A. Banks, B.A. Baldwin, D.J. McGoldrick, and S.M. Blankenship. 1995. Pedigree Analysis of Captive Broodstock for an Endangered Chinook Salmon, Using Tandem-Repeat DNA Polymorphisms. Conserv. Biol.

Nielsen, Jennifer L., C. Gan, and W.K. Thomas. 1994. Differences in Genetic Diversity for Mitochondrial DNA Between Hatchery and Wild Populations of *Oncorhynchus*. Can. J. Fish. Aquat. Sci. 51(Suppl. 1):290-297.